WATER BALANCE, HOST UTILISATION, AND MASS REARING IMPROVEMENTS OF THE COCKROACH OOTHECAL PARASITOIDS, *Aprostocetus hagenowii* (HYMENOPTERA: EULOPHIDAE) AND *Evania appendigaster* (HYMENOPTERA: EVANIIDAE)

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BY

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ABSTRAK

Tesis ini menumpu kepada keseimbangan air, penggunaan perumah and peningkatan kultur massa *Aprostocetus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae) dan *Evania appedigaster* (Linnaeus) (Hymenoptera: Evaniidae), dua parasitoid ooteka lipas Amerika *Periplaneta americana* (Linnaeus) (Blattidae: Dictyoptera). *Evania appendigaster* mempunyai kebolehtelapan kutikal yang lebih tinggi daripada *A. hagenowii.* Kesemua parasitoid, kecuali jantan *A. hagenowii*, mempunyai kadar kehilangan jumlah kandungan air dalam badan yang sama. Kelanjutan usia kesemua parasitoid, kecuali jantan *E. appendigaster*, berkurangan dengan penurunan kelembapan relatif (RH) tetapi fenomena tersebut tidak berlaku antara parasitoid-parasitoid yang diberi gula. Dalam kebuk yang mengandungi satu gradien RH 44–87%, kedua-dua jantina *E. appendigaster* menunjukkan kecenderungan kepada kebuk yang paling lembap. Bagi *A. hagenowii*, betina menunjukkan kecenderungan kepada kedua-dua kebuk yang paling kering dan yang paling lembap dan jantan menunjukkan kecenderungan kepada kebuk yang paling kering. Pengaruh umur ooteka dan suhu malar ke atas sejarah kehidupan *A. hagenowii* telah dikaji. *Aprostocetus hagenowii* mampu berkembang dalam ooteka-ooteka mencapai sehingga 72.9% daripada jumlah jangka waktu perkembangan embrio tanpa sebarang kesan ke atas ciri-ciri kecergasan mereka. Ambang suhu bawah, atas dan

optimum suhu adalah 9.5, 34.2 and 31.1 °C, masing-masing. Suhu tidak memeberikan ciri reproduksi betina tetapi menpengaruhi activti parasitisme melalui masa. Kesan umur ooteka ke atas kanibalisme ooteka pada *P. americana* dan pengaruhannya ke atas pemilihan umur ooteka dan kecergasan dalam *E. appendigaster* telah diperiksa. *Periplaneta americana* lebih cenderung memakan ootheca berumur 1 hari berbanding dengan ooteka berumur 10–40 hari. Walaubagaimanapun, parasitoid betina lebih cenderung memparasit ooteka berumur 1 hari daripada yang berumur 10–40 hari. Kadar kemunculan adalah lebih tinggi dan masa pengendalian lebih pendek pada ooteka berumur 1 hari berbanding dengan yang lebih tua. Bagi progeni parasitoid, masa perkembangan meningkat, dan saiz badan dan jangka hayat berkurangan dengan umur ooteka. Keputusan-keputusan ini mecadangkan bahawa betina *E. appendigaster* mendagangkan kemandirian progeni untuk keuntungan kecergasan bagi mereka sendiri dan progeny mereka. Masa pemanasan minium yang diperlukan untuk membunuh ooteka telah ditentukan dan kesesuaiannya sebagai perumah setelah disimpan dalam berberapa keadaan yang berbeza (secara hidup, dibunuh dengan pemanasan dan disimpan sejuk selama 0.5–4 bulan pada 4 °C) untuk pembiakan parasitoid *A. hagenowii* dan *E. appendigaster* telah dikaji. Pemanasan pada 48 °C selama 45 min membunuh ooteka-ooteka yang pelbagai umur. Rawatan pemanasan meningkatkan kesesuaian ooteka berumur 4 minggu sebagai perumah untuk pembiakan parasitoid. Bagi *A. hagenowii*, kadar penetasan yang rendah telah didokumenkan antara ooteka-ooteka berumur 3–4 minggu yang telah disimpan sejuk. Bilangan progeni yang dihasilkan daripada ooteka berumur 1–2 dan 3-4 minggu berkurangan setelah disimpan sejak 3–4 dan 0.5–4 bulan, masing-masing. *Evania appendigaster* mempunyai kadar penetasan yang rendah dalam ooteka yang disimpan sejuk.

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ABSTRACT

This thesis focuses on the water balance, host utilization and mass rearing improvements of *Aprostocetus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae) and *Evania appeindgaster* (Linnaeus) (Hymenoptera: Evaniidae), two ootheca parasitoids of the American cockroach, *Periplaneta americana* (Linnaeus) (Blattidae: Dictyoptera). *Evania appendigaster* had higher cuticular permeability than *A. hagenowii*. All of the parasitoids, except *A. hagenowii* males, had similar percentage total body water loss rate. The survival times of all the parasitoids, except *E. appendigaster* males, reduced with decreasing relative humidity (RH) but this phenomenon did not occur among sugar-fed parasitoids. In chambers with a 44–87% RH gradient, both sexes of *E. appendigaster* preferred the wettest chamber. For *A. hagenowii*, females preferred both the driest and the wettest chambers and males preferred the driest ones. The effect of ootheca age and constant temperature on the life history of *A. hagenowii* were studied. *A. hagenowii* could successfully develop in oothecae attaining up to 72.9% of the total embryonic development time without any effect on their fitness traits. The lower, upper and optimal temperature thresholds were 9.5, 34.2 and 31.1 °C, respectively. Temperature did not affect females' reproductive traits but did influence parasitism activities over time. The effect of ootheca age on ootheca cannibalism in *P. americana* and its influence on ootheca age selection and

fitness in *E. appendigaster* was examined. *Periplaneta americana* differentially cannibalized 1 d versus 10–40 d old oothecae. However, parasitoid females preferred to parasitize 1 d old than 10–40 d old oothecae. The emergence rate was greater and the handling time was shorter on 1 d old compared to older oothecae. For parasitoid progeny, development time increased and body size and longevity decreased with ootheca age. These results suggest that *E. appendigaster* females traded progeny survival for fitness gains for themselves and their progeny. The minimum time of heating required to kill oothecae was determined and their suitability as hosts after different storage conditions (live, heat killed, and stored for 0.5–4 months at 4 °C) for rearing parasitoids *A. hagenowii* and *E. appendigaster* was investigated. Heating at 48 °C for 45 min killed oothecae of all ages. Heat treatment improved the suitability of 4-week-old oothecae as hosts for parasitoid rearing. For *A. hagenowii*, reduced emergence rates were documented among cold-stored 3–4-week-old oothecae. The number of progeny produced in 1–2-week-old and 3–4-week-old oothecae decreased after cold-stored for 3–4 and 0.5–4 months, respectively. *Evania appendigaster* had low emergence rates in cold-stored oothecae.

CHAPTER ONE

INTRODUCTION

The American cockroach, *Periplaneta americana* (Linnaeus), is one of the major cosmopolitan pest cockroaches of economic and medical importance (Cornwell, 1968; Lee, 1997, 2007; Rust, 2008). In the human living environment, this species lives in large numbers in moist and warm habitats such as sewers, drainage systems, and rubbish chutes (Lee and Ng, 2009; Rust et al., 1991; Tee et al., 2011a) and consequently provides reservoir populations to fuel indoor and outdoor infestations (Robinson, 2002). Because it inhabits areas associated with human waste and disease organisms, *P. americana* had been reported to be a potential mechanical vector of pathogenic bacteria, viruses, fungi, protozoans, and helminthes (Lee, 1997; Roth and Willis, 1957). Cockroach allergens also contribute to the occurrence of asthma and allergy among sensitized individuals (Gore and Schal, 2007).

Chemical control is widely used for cockroach management. However, awareness of the adverse effects of insecticide exposure in the human environment has prompted research of possible alternative approaches. Biological control of cockroaches using oothecal parasitoid wasps is an alternative approach that warrants further study. Because cockroach eggs are enclosed within oothecae and deposited in hidden locations (Rau, 1943; Yeh, 1995), they often are not eliminated by insecticide treatment. Use of oothecal parasitoid wasps is one way to control this immobile stage.

Evania appendigaster (Linnaeus) (Hymenoptera: Evaniidae) and *Aprostocetus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae) are ootheca parasitoids of the American cockroach. *Evania appendigaster* is a solitary species (only one wasp emerge from an ootheca) whereas *A. hagenowii* is a gregarious species (numeruous wasps produced per ootheca). Females lay their eggs inside oothecae in which 16 cockroach eggs are enclosed in two rows. The immature stages of both parasitoid wasps consume all of the cockroach eggs and take 32–46 d (25–30 °C) to develop before they chew an exit hole on the surface of the ootheca to emerge as adult wasps (Bressan-Nascimento et al., 2010; Cárcamo et al., 2013). *Aprostocetus hagenowii* is smaller in body size (1.3–2.1 mm in length) but a superior larval competitor compared to *E. appendigaster* (6.5–8.0 mm) (Narasimham, 1984; Kumarasinghe and Edirisinghe, 1987).

The use of ootheca parasitoids for cockroach control faces several constraints: (1) limited information about how environmental factors (humidity and temperature) influence the foraging and fitness of ootheca parasitoids. Several field release studies of ootheca parasitoids had been conducted at different geographical regions (tropics to subtropic) and microhabitats (e.g., treehole habitats, plumbing chases, sewer systems, around and inside buildings). These studies reported mixed results (parasitism rates on oothecae range 0–100%). Knowledge of the environmental factors on parasitoids' foraging and fitness is critical to improve their use as biological control agents in cockroach control programs. (2) Host utilization pattern of each ootheca parasitoid is poorly understood. Host age is one important criterion of host quality that affects parasitoid fitness. It is important to assess the effect of ootheca age on parasitoid fitness as this would help to improve the rearing of the parasitoids and their use for cockroach

control, such as estimation of the parasitism dynamics in the field, release schedule and strategies (single or multiple species). (3) Methods for mass rearing of these ootheca parasitoids are not adequately developed, and to date an artificial diet is not available. Because *P. americana* has a slow ootheca production rate (females produce an average of 1–2 oothecae per week for their entire lifespan) (Gould and Deay, 1938; Roth and Willis, 1956), limited numbers of cockroach oothecae can be obtained from laboratory rearing of cockroaches. Therefore, finding a way to stock pile oothecae may assist in the mass production of ootheca parasitoids for use in biological control of cockroach populations.

Coexistence of competing parasitoids parasitizing the same host species can be facilitated by niche partitioning. Niche partition (temporal or spatial) can be mediated when differences in the intrinsic biological attributes between competing species results in differences in their abilities to exploit host of varying distribution, such as differences in competitive and dispersal abilities (Lei and Hanski, 1998), ovipositor length (Hanks et al., 2001), host detection behavior (van Dijken and van Alphen, 1998) and responses to extrinsic environmental factors (humidity, temperature, nutrients, etc.) (Amarasekare, 2003; Sorribas et al., 2010). Both parasitoid species are widespread and commonly found coexisting in areas where *P. americana* occurs (Roth and Willis, 1960; Piper et al., 1978; Narasimham and Sankaran, 1979; Kumarasinghe and Edirisinghe, 1991). This coexistence of ootheca parasitoids provide an excellent multiple-parasitoids-host model to study the biological differences between competing species and, thus, allows the key features that are unique to the ecological success of each parasitoid species to be identified. This would also benefit the use of these parasitoid for cockroach control.

This study focuses on the host utilization pattern of *A. hagenowii* and *E. appendigaster* in relation to ootheca age and extrinsic environmental factors (humidity, temperature and sugar food resources), and their mass rearing improvements through the following objectives:

1. To study the water balance profiles, humidity preference and survival of both parasitoids in order to understand the impact of desiccation on forming their ecological niche.

2. To investigate the effect of ootheca age on the effect of ootheca age on cannibalism in the *P. americana* and its effect on host age selection and fitness of *E. appendigaster*.

3. To examine the influence of ootheca age and temperature on the immature development and adult fitness of *A. hagenowii*.

4. To assess the feasibility of using cold-stored *P. americana* oothecae for rearing *A. hagenowii* and *E. appendigaster*.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Periplaneta americana*

Periplaneta americana is one of the major pest cockroach species of medical and economic importance in tropical and subtropical regions (Cornwell, 1968). It has been found associated with pathogenic bacteria, viruses, fungi, protozoans and helminthes (Roth and Willis, 1957, 1960; Lee, 1997; Lee and Ng, 2007). Seven allergens have been identified in *P. americana* and these could potentially lead to household asthma and allergy (Gore and Schal, 2007).

Adult cockroaches are 28–44 mm in length and shinning dark-brown in color with a pale-yellow strip around the edge of the pronotum (Plate 2.1) (Cornwell, 1968). Adult males can live for 125–362 days whereas adult females have a longer lifespan of 125–706 days (Rust, 2008). Females produce 1–2 oothecae during their peak reproductive period and a total of 10–84 oothecae during their entire lifespan (Gould and Deay, 1938; Roth and Willis, 1956). Ootheca is an egg case in which 12–16 cockroach eggs are enclosed (ootheca generally refers to both the egg case and enclosed eggs) (Roth, 1968; Bell and Adiyodi, 1981). Oothecal shell protects developing embryos from desiccation, physical damage, and disease infections (Roth and Willis, 1955; Provine, 1981). Ootheca is soft and reddish-brown in color upon newly laid, and is hardened and darkened within the next few days after deposition. Incubation time of *P. americana* eggs range from 40 to 69 days at 20–30°C

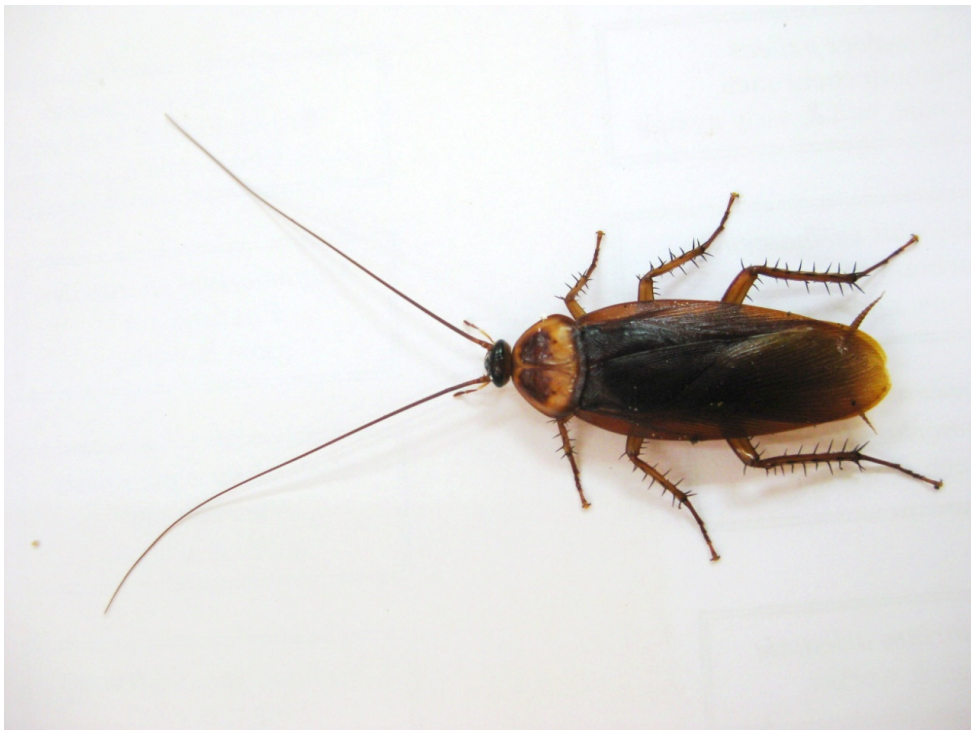

Plate 2.1 Adult male of *P. americana.*

(Bressan-Nascimento et al., 2008). Nymphs undergo 6–14 molts in 150–450 days (temperature-dependent) before maturing into the adult stage (Cornwell, 1968).

American cockroaches frequently shelter in warm and moist microhabitats inside and around buildings such as tree holes, sewers, crack and crevices, bin-chute and drainage systems (Appel, 1986; Suiter et al., 1998; Lee and Ng, 2009; Tee et al., 2011a). This is partly due to their vulnerability to desiccation as Appel et al. (1983) have shown that this species has a hygric-typed water loss rate (1.82% h^{-1}). Cockroaches aggregate in shelters in groups, which is facilitated by attraction to aggregation pheromone presented in their feces (Roth and Cohen, 1973; Bell, 1981). This group living enhances development, sexual maturation, survival, sexual maturation and foraging efficiency for food and shelter (reviewed by Bell et al., 2007 and Lihoreau et al., 2012). However, group living also incur cannibalism among group members. There is little information about why cockroaches exhibit cannibalistic behaviors but hypotheses have been proposed that (1) it occurs as a by-product of their opportunistic and omnivorous feeding habit, where cannibals take the advantage of consuming vulnerable conspecifics (oothecae, young and individuals that are newly molted, injured and weak) and cannibalism frequency varies depending on the cockroach density and food availability (quality and quantity); and (2) it serves to recover and recycle essential nitrogenous resources back into cockroach aggregation (Ross and Mullins, 1995; Nalepa and Bell, 1997; Bell et al., 2007). Cannibalism of oothecae in blattid pest cockroaches is commonly observed (Roth and Willis, 1960; Gordon et al., 1994). However, studies of the benefits of ootheca cannibalism in cockroaches are limited. Nalepa and Mullins (1992) estimated that adult female wood cockroach, *Cryptocercus punctulatus* Scudder, could recover up to 58.7%

of the nitrogen she invested into her brood inside a single ootheca by eating the oothecal shell after hatching. The oothecal shell of *P. americana* consists of 87% protein (Kramer et al., 1991) but little is known about the nutritional benefits of eating ootheca in this species.

2.2 *Aprostocetus hagenowii*

Aprostocetus hagenowii is a gregarious parasitoid wasp of cockroach oothecae. Its cockroach hosts include *P. americana*, *Periplaneta australasiae* (Fabricius), *Periplaneta fuliginosa* (Serville), *Blatta orientalis* (Linnaeus), *Parcoblatta* spp., *Eurycotis floridana* (Walker) and *Eurycotis biolleyi* Rehn (Roth and Willis, 1960; Lebeck, 1991).

Adult wasps are black in color and 1.8–2.0 mm in length (Plate 2.2) (Narasimham, 1984). The lifespan of males and females reared without foods are 1.5–1.8 and 3.8–5.9 days, respectively. However, males and females can live for an extended time if fed with carbohydrate-based foods (Narasimham, 1984). In the laboratory, *A. hagenowii* given mucilage of *Hibiscus rosa-sinensis* L. and nectar of *Leucas aspera* (Wild.) had longer lifespan than those reared without foods (Narasimham, 1984). This suggests that, similar to many other parasitoid wasps, they may feed on floral food resources in natural environments (Jervis et al., 1993). Females have a pair of ovaries each consists of 8–12 ovarioles. Females have been documented to possess 45 mature eggs in their ovaries upon emergence and continue to mature more eggs in the following days (Zhang et al., 2010).

Plate 2.2 *Aprostocetus hagenowii* female attempts to drill and insert its ovipositor into a *P. americana* ootheca.

Females can parasitize 1–5 oothecae in their entire lifespan, producing an average of 103 progeny (range 50–139) (Roth and Willis, 1954). Females lay a large portion of their lifetime total number of mature eggs into the first ootheca they encountered and smaller numbers of eggs into other oothecae they found subsequently (Roth and Willis, 1954). An ootheca that has been parasitized by an oviposition-inexperienced (virgin) female can produce 45–96 progeny with a female-biased sex ratio of 0.07–0.09 (proportion of males) (Suiter et al., 1998; Tee et al., 2010). Developmental time of *A. hagenowii* varies between 22 and 90 days (Roth and Willis, 1960). An ootheca can produce a greater number of progeny (up to 261 wasps) if it is superparasitized by many females (Lebeck, 1991). *Aprostocetus hagenowii* is a facultative hyperparasitoid of *E. appendigaster*. It can successfully develop in an ootheca containing a developing *E. appendigaster* of up to 32-days-old (Kumarasinghe and Edirisinghe, 1987). Piper et al. (1978), in their survey of the ootheca parasitoids around residential properties in Texas and Louisiana, documented that 96.5% of the 113 parasitized oothecae produced *A. hagenowii* and the remaining oothecae had *E. appendigaster* emerged.

Saliva used by *P. americana* females to glue ootheca to a substrate had been found to serve as a host recognition cue in *A. hagenowii* (Vinson and Piper, 1986). In addition, one short-range volatile hydrocarbon, identified as 6,9-heptacosadiene, had been detected from *P. americana* frass, oothecae and females, which serves as a kairomone that attracts *A. hagenowii* (Suiter et al., 1996). This hydrocarbon consists of a large portion of cuticular hydrocarbons of *P. americana* and is absent in *P. australasiae*, *Periplaneta brunnea* Burmeister, and *P. fuliginosa* (Saïd et al., 2005). This likely suggests the role of 6,9-

heptacosadiene in mediating the preference of *A. hagenowii* for *P. americana* over other blattid cockroach species (Roth and Willis, 1954).

2.3 *Evania appendigaster*

Evania appendigaster is a solitary ootheca parasitoid of blattid pest cockroaches which include *P. americana*, *P. australasiae* and *B. orientalis*. It is found in tropical and subtropical regions (Roth and Willis, 1960). Adult wasp is black in color and 6.5–8.0 mm in body length (Plate 2.3). Wasps in the family Evaniidae can be easily recognized as they have a laterally-flattened abdomen, which is attached to the top of the propodeum by a petiole. This unique abdominal structure resembles a flag and, thus evaniids are collectively known as ensign wasps (Stange, 2010).

Adult wasps can live for 1–2 weeks without foods and can extend their lifespan to 3–6 weeks when fed with carbohydrate-based foods (Yeh and Mu, 1994a; Fox and Bressan-Nascimento, 2006). Females have a pair of ovaries each bearing an average of six ovarioles. Females have a total of 12 mature eggs with each ovariole containing one mature egg upon emergence (Yeh and Mu, 1994a). Females can produce up to 23 progeny during their entire lifespan (Fox and Bressan-Nascimento, 2006).

Oviposition by a female on an ootheca takes an average of 1342 seconds, which involves a sequence of behaviors; (1) host contact and probing using antennae, (2) unsheathe ovipositor to probe and find a suitable drilling site, (3) female remains quiescence with its tip of ovipositor touching the surface of ootheca, (4) drilling and insertion of ovipositor, (5) oviposition, (6) withdrawal of ovipositor and leaving the

Plate 2.3 *Evania appendigaster* female remains quiescence on an ootheca with the tip of its ovipositor touching the surface of the ootheca.

ootheca (Yeh and Mu, 1994b). Of these oviposition behaviors, the quiescent stage takes up 70% (ca. 1000 s) of the time budget for oviposition. Yeh and Mu (1994b) proposed that females might be releasing protein-denaturing enzymes during the quiescent stage to facilitate the insertion of the ovipositor into ootheca.

Evania appendigaster females normally lay one egg into an ootheca (≤ 6% self-superparasitism) and only one wasp is produced from an ootheca in the case of superparasitism (Bressan-Nascimento et al., 2010; Fox et al., 2012). Developmental time from egg to adult takes 36–45 days at 25–30°C (Bressan-Nascimento et al., 2010). After hatching, the larva undergoes two molts and consumes all the cockroach eggs before reaching the pupal stage. Upon emergence, parasitoid chews an exit hole through the ootheca shell to emerge as an adult wasp (Fox et al., 2012). Mating and oviposition can occur immediately after emergence. Females are monandrous (mating once with just one male) whereas males are polygynous (mating with many female mates) (Yeh and Mu, 1994a).

2.4 Interspecific competition and coexistence of competing species in parasitoid wasps

Parasitoid wasps often compete with other heterospecific parasitoids for the same host resources in the same habitat. This interspecific competition can occur among parasitoid larvae that compete for limited resource from a single host (intrinsic competition) and between female foragers that search for and parasitize the same host resources (extrinsic competition) (Cusumano et al., 2012). In natural ecosystems, it is

common that more than one competing parasitoid species can coexist. Typically only one parasitoid species can survive from an intrinsic competition for a single host resource (Cusumano et al., 2012; Mills, 2006). The outcome of such competition is affected by several mechanisms such as physiological suppression, physical attack, sequence of and time interval between ovipositions (Vinson and Hegazi, 1998; Mills, 2006; Uka et al., 2006; Bai et al., 2011; Cusumano et al., 2012). Thus, whether competing parasitoids can co-exist cannot be determined based solely on the outcome of intrinsic competition.

Several studies demonstrated mechanisms that shape the coexistence of parasitoid wasps, such as responsiveness to host productivity gradient (Amarasekare, 2000), spatial and temporal niche partitioning (Hanks et al., 2001; Sorribas et al., 2010). Among these mechanisms, spatial niche partitioning is commonly found to facilitate coexistence of parasitoids (Mills, 2006). Spatial niche partitioning can be mediated when parasitoids differ in their biological attributes to exploit spatially separated hosts. For example, difference in ovipositor length between *Syngaster lepidus* Brullé and *Callibracon limbatus* (Brullé) had resulted in spatial partitioning of the larvae of eucalyptus longhorned borer *Phoracantha semipunctata* Fabricius concealing inside tree bark of varying depth; *S. lepidus* with a longer ovipositor (10.1 mm) than *C. limbatus* (8.6 mm) was able to parasitize larvae concealed in thicker tree bark (Hanks et al. 2001). In another study, Lei and Hanski (1998) demonstrated spatial partitioning in parasitoids is mediated by trade-off between dispersal and competition ability. In their study, parasitoid species that was inferior larval competitor but a good disperser efficiently utilized isolated host patches, which might not have been colonized by another competing species that was a superior larval competitor but a poor disperser.

2.5 Water balance in insects

Insects face the challenge of maintaining a balance body water in relation to the desiccation stress of their habitats. Insects living in a drier area have lower water loss rates than those found in more humid environments; they can be classified based on the water loss rates (% body water loss h^{-1}) introduced by Hadley (1994) as xeric (< 0.8% h^{-1}), mesic (0.8–2.0% h^{-1}) and hygric species (> 2.0% h^{-1}). Insects lose their body water through evaporation at the cuticle surface, respiration, defecation and secretion. Among these water loss routes, cuticular water loss is a major water loss route that accounts for > 80% of the total body water loss in most insects (Hadley, 1994; Gibbs and Rajpurohit, 2010). This is because (1) cuticular water loss occurs as a function of the body surface area and (2) insects that are small in size have a large body surface area relative to body volume for body water to be lost through the cuticle. Thus, cuticular permeability and body size are two interacting factors that influence water loss rates of insects.

Cuticular lipids at the surface of the cuticle have been recognized as the most effective barrier in preventing cuticular water loss in most insects (Gibbs and Rajpurohit, 2010). Cuticular lipids mainly consists of hydrocarbons. The quantity and composition (e.g., chain length, saturation and numbers of methyl-branching) of cuticular hydrocarbon account for differences in water loss rates of insects at the intra- and interspecific levels (Gibbs 1998; Parkash et al., 2008a; Gibbs and Rajpurohit, 2010). For example, under an extended desiccation selection period, *Drosophila melanogaster* Meigen populations have been found to develop a lower water loss trait, partly due to their longer-chained cuticular hydrocarbons compared to those of control populations (Gibbs et al. 1997). Besides

cuticular lipids, melanization of cuticle also affects cuticular water loss (Parkash et al. 2008a, 2008b). Cuticle is hardened and darkened during melanization process, which results in a layer of more compact and water-proofed cuticle (Gibbs and Rajpurohit, 2010). For example, Parkash et al. (2008b) demonstrated that darker *Drosophila immigrans* had lower water loss rates than those of lighter flies, even though both fly populations showed no difference in the quantity of cuticular lipids.

Body size has been found responsible for the intra- and interspecific variation in desiccation resistance in insects (Hood and Tschinkel, 1990; Addo-bediako et al., 2001; Kaersgaard et al., 2004; Schilman et al., 2007; Yoder et al., 2010). It is expected that insects of smaller sizes are vulnerable to water loss than those of bigger sizes because smaller body size means larger body surface area/body volume for cuticular water loss. Thus, increase in body size is one way for insects to be less susceptible to desiccation (Danks, 2000; Benoit and Denlinger, 2010). For example, desiccation resistance was positively correlated with body size in arboreal and terrestrial ant species (Hood and Tschinkel, 1990). In a study of the foraging activity of ants on the forest floor, Kaspari (1993) documented that small ant species forage primarily in moist environment in closed canopy whereas large ant species could expand their foraging territory into drier habitats in open canopy. In addition, two sympatric congeneric subterranean termites with similar cuticular permeability were documented to adopt distinct foraging strategies due in part to difference in their body size; bigger-sized *Macrotermes carbonarius* (Hagen) was documented to be able to forage above ground in open-air condition during wet seasons while smaller-sized *M. gilvus* (Hagen) foraged mainly in concealed conditions (underground tunnels and mud tubes) and rarely in open-air environment (Hu et al., 2012).

Most insects possess body water content of 65–75% of their fresh weight (Hadley, 1994; Danks, 2000). Total body water content is one physiological feature commonly examined in the studies of desiccation resistance in insects. Conserving high body water content is one strategy for insects to reduce desiccation risk (Gibbs et al., 1997; Danks, 2000; Schilman et al., 2007). In a study of physiological adaptations of insects towards desiccation stress, Gibbs et al. (1997) showed high body water content (approximately 30% more) was a trait selected for *D. melanogaster* subjected to desiccation selection, which render these flies less susceptible to desiccation than that of control flies. Yoder et al. (1998) also documented that the parasitoid *Ageniaspis citricola* (Logvinovskaya) had a high body water content of 75%, which was at the upper range of the average value for most insects.

2.6 Factors influencing host selection and foraging of parasitoids

Host quality is one important determinant of parasitoid fitness as parasitoid larvae depend solely on a single host for acquisition of nutrient. Besides host species and size variation, host quality is influenced by host age as host undergoes biochemical, physiological and morphological changes during the course of its development. These changes affect both the fitness of ovipositing females and their progeny developing on/inside the host. Depending on host types (eggs, larvae, pupae or nymphs) and parasitoids' development modes (idiobiont: hosts are sessile and do not grow in size after parasitism; koinobiont: hosts are mobile and continue to grow after parasitism), parasitoids differ in their host-age related fitness trade-offs during host-parasitoid interaction.

For egg and pupal parasitoid wasps, host quality generally decreases with age because host resources do not increase in size and are converted into more complex host tissues (e.g, well-developed embryos and sclerotized pupae), which become less accessible and suitable for parasitoid development. Thus, egg and pupal parasitoids may take longer time to develop and experience reduced body size, longevity and fecundity when they develop in older hosts. Vinson (1998) has proposed three oviposition strategies female egg and pupal parasitoids commonly used to maximize their fitness in relation to host-aged issues (Vinson, 1998); (1) parasitoids should oviposit in only young hosts, which can be facilitated by acceptance of only young hosts for oviposition through assessment of host's chemical and physical cues, presence before host eggs are laid (phoretic behavior, attraction to host sex or aggregation pheromones), and attraction towards female oviposition cues, such as oviposition secretions. Studies of host preference in egg and pupal parasitoids have shown that females exhibited oviposition preference for hosts of young or/and intermediate age compared to old hosts, via either innate preference or learning of host cues (Kouamé and Mackauer, 1991; Ueno, 1997; Chow and Mackauer, 1999; Godin and Boivin, 2000). For example, Godin and Bivin (2000) found that, in a multiple-choice condition, 70% of the 42 species and strains of trichogrammatid egg parasitoids prefer to parasitize young and intermediate-aged over old host eggs. Vinson (1998) postulated that the amount of host's volatile compounds may change over time. Female wasps may make use of these changes in volatility in their search for newly-laid hosts; (2) parasitoid larvae should increase their development rates when they are being laid in hosts of older ages. However, little is known about whether parasitoid larvae adjust their development rate according to host age. Studies of egg and pupal parasitoids commonly demonstrate that development time of parasitoid wasps increases with host age

due to a longer time needed to consume and assimilate well-developed host tissues (Otto and Mackauer, 1998; Hirose et al., 2003); and (3) parasitoids can arrest or delay host development and breakdown host tissue, rendering old host suitable for parasitoid larval development. It has been demonstrated that newly-hatched larva of *Trichogramma pretiosum* Riley consumed host embryos and yolk, which had been extraorally digested by factors female wasp injected during oviposition (Strand, 1986; Pennacchio and Strand, 2006; Strand, 2014). In another study, Strand et al. (1986) discovered that female of the scelionid egg parasitoid *Telenomus heliothidis* Ashmead release factors that arrest host embryogenesis while teratocytes released from hatching egg of parasitoid facilitate extraoral digestion of host embryo. Zhou et al. (2014) further shown that *Telenomus podisi* (Say) could even successfully develop in hosts 24 h prior to its hatching (incubation time takes six days) without incurring detrimental effects on its fitness traits (survival, body size, fecundity and sex ratio, except developmental time).

For idiobiont larval parasitoids, host larvae increase in size with age. Thus, parasitoid larvae developing in older and larger hosts attain greater body size (and associated fitness correlates) than those utilizing young and small hosts (Liu et al., 2011). However, female parasitoids need to invest more energy, time and venom to parasitize an older and larger host, which costs them a greater handling time and risk of injury/death and reduced parasitism success rate and fecundity (Wei et al., 2014). Consequently, there are trade-offs between fitness traits in relation to host age (Liu et al., 2011; Wei et al., 2014). By contrast, koinobiont parasitoid larvae could delay development when they are deposited in smaller hosts in order to benefit a greater nutrient pool accumulated in later

host stages of larger sizes (Harvey and Strand, 2002; Colinet et al., 2005; Jenner and Kuhlmann, 2006).

Female parasitoids also assess the risk of predation and hyperparasitism during host foraging and selection. Avoidance of high-risk host patches through detection of predators/hyperparasitoids or their traces and switch to attack enemy-free or low-risk patches are one way for parasitoid wasps to overcome predation or hyperparasitism risk (Höller et al., 1993; Taylor et al., 1998). However, because majority of parasitoid wasps are time-limited and have limited reproductive resources (due to lack of lipogenic ability, and lipids, as an essential nutrient for egg production, are accumulated during parasitoid larval development), females are expected to exhibit adaptive host foraging strategies in order to maximize their reproductive fitness. In the context of host-parasitoid-predation/hyperparasitoid interaction, adaptive host foraging in parasitoid wasps often involves trade-off between survival and fitness of parasitoid. For example, females of the aphid parasitoid *Pauesia silvestris* Stary were found to optimize host utilization by shifting to attack hosts of lower quality when hosts of higher quality were distributed in microhabitats with a high hyperparasitism risk (Völkl and Kroupa, 1997). This optimal host foraging had resulted in a greater overall fitness gain for *P. silvestris*. In another study, Ayal and Green (1993) reported that female aphid parasitoid *Diaeretiella rapae* (M'intosh) optimized the number of aphids to be parasitized per aphid colony based on aphid density and the frequency of hyperparasitoid visits. This optimal ovipsotition strategy maximizes the number of parasitoids emerged per aphid colony because hyperparasitoids spent less foraging time for aphid colonies with low density of *D. rapae*-infected aphids.

Besides adult female wasps, parasitoid larvae also exhibit adaptations to reduce mortality risk due to predation and hyperparasitism. For example, Harvey and Strand (2002) reported that a distinct difference in developmental trait among koinobiont parasitoids in the families Icheneumonidae and Braconidae, which attack hosts of different feeding ecology. They found that koinobiont parasitoid larvae would prolong their development time in small host larvae that feed in concealed environment so that they could obtain more nutrients along host development. By contrast, koinobiont parasitoid larvae that develop in host larvae feeding at exposed environments would trade off potential gain in body size for rapid development, which minimize their time window of vulnerability to predation and hyperparasitism.

2.7 Rearing of *A. hagenowii* and *E. appendigaster* for biological control of cockroaches

Yeh et al. (1995) described an efficient way to collect oothecae from *P. americana* cultures for use in the maintenance of cockroach cultures and parasitoid rearing. They demonstrated that oothecae can be collected from a piece of styrofoam board, which was placed into a rearing container of *P. americana* females. This method takes the advantage of the oviposition concealment behavior of *P. americana* female, where it tends to chew out a cavity on a soft and thick substrate and cover its ootheca inside the cavity with debris held in place with its saliva (Rau, 1943; Bell and Adiyodi, 1981; Yeh, 1995).

In biological control program, parasitoid rearing methods that produce female-biased clutches are desirable as females are responsible to suppress insect pest populations.

Hagenbuch et al. (1988) developed a rearing method to produce *A. hagenowii*. In their study, a parasitoid production unit was constructed using a chamber, which consisted of a dark and a light side; the dark side was used to house parasitized oothecae so that, upon emergence, parasitoids would be attracted to the light side (due to its positive phototactic response), where 30–40 healthy oothecae were exposed for parasitism. Although this method provides a convenient way to prepared *A. hagenowii*-parasitized oothecae, the resultant sex ratio (number of males/number of parasitoid produced) of parasitoids per ootheca was less female-biased (0.47). Several studies have shown that sex ratio of *A. hagenowii* produced from an ootheca could be greatly improved (ranged 0.07 to 0.12) when oothecae were exposed individually to a female wasp for oviposition (Suiter et al., 1998; Tee et al., 2010). This individually exposure method is time-consuming but it produces *A. hagenowii* females with a larger body size and a longer lifespan than those produced from superparasitized ootheca (Narasimham, 1984).

In biological control programs, use of killed hosts for parasitoid rearing is one important step because it (1) eliminates the needs to handle individuals hatching or emerging from unparasitized hosts into rearing cages, (2) prevents accidental releases of insect pests from unparasitized hosts into the field, and (3) retains hosts at stages optimum for parasitoid development (Suiter et al., 1998; Geden and Kaufman, 2007; Tee et al., 2010). Techniques used to kill host insects without compromising their suitability as hosts for parasitoid rearing include exposure of hosts to irradiation (e.g., ultraviolet (UV), gamma-ray, X-ray) and high/ low temperatures (Suiter et al., 1998; Moreno et al., 2009; Cancino et al., 2012).

Studies of the rearing improvement showed that gamma-irradiation could terminate embryonic development of *P. americana* and these-irradiated oothecae were as suitable as live oothecae for rearing *A hagenowii* and *E. appendigaster* (Suiter et al., 1998: Hwang and Chen, 2004). Similarly, heating of oothecae at 48–50 °C provides an economical way to produce killed oothecae for rearing *A. hagenowii* and *E. appendigaster* but long heating time would reduce the quality of oothecae for parasitoid rearing (Hwang and Chen, 2004; Tee et al., 2010). Tee et al. (2010) showed that a minimal heating time of 30 min at 48 °C was sufficient to terminate embryonic development of 1–2-days-old oothecae. By contrast, exposure of oothecae to freezing temperatures (–16 and –20 °C) can kill *P. americana* eggs but such killed-oothecae are not suitable as hosts for *A. hagenowii* and *E. appendigaster* as due to low emergence rates (Suiter et al., 1998: Hwang and Chen, 2004). Alternatively, Bressan-Nascimento et al. (2008) demonstrated that oothecae subjected to a chilling temperature of 0–5 °C terminated *P. americana* embryogenesis without compromising their quality for rearing both *A. hagenowii* and *E. appendigaster*. However, methods to increase the shelf-life of these killed oothecae are not adequately developed. Studies on other parasitoids have shown that storage at low temperatures can be a viable way to stock-pile hosts for mass-rearing and field releases of parasitoids (Chen and Leopold, 2007; Geden and Kaufman, 2007).

2.8 Biological control efficacy of *A. hagenowii* and *E. appendigaster*

Aprostocetus hagenowii and *E. appendigaster* are prevalent among natural cockroach populations. Surveys of field collected oothecae showed that parasitism of *A. hagenowii* rated 16–48% in *P. americana*, 22–84% in *P. fuliginosa* and 12.5% in *B.*

orientalis (Cameron, 1955; Fleet and Frankie, 1975; Kanayama et al., 1976; Piper et al., 1978; Narasimham and Sankaran, 1979) whereas parasitism of *E. appendigaster* was 29% in *P. americana* (Cameron, 1957). However, placement of sentinel oothecae inside sewer manholes, one of the primary breeding sites of *P. americana*, failed to detect the presence of *A. hagenowii* (Reierson et al., 2005; Tee et al., 2011b).

Several studies have been conducted to evaluate the biological control efficacy of *A. hagenowii* in indoor room condition and at natural cockroach habitats. These studies reported varying parasitism rates. Releases made in room conditions generally produced the most promising results. For example, Roth and Willis (1954) documented that a release of 1000 female wasps into a laboratory room could achieve up to 83% parasitism rate. Similarly, Hagenbuch et al. (1989) reported high parasitism rates (95–98%) for an evaluation, where 300 female wasps were released weekly into a simulated room.

In field evaluations, weekly releases of *A. hagenowii* at plumbing chases, crevices around buildings and sewer systems resulted in 18.2–30.6%, 4.2–37.5% and 5.6–24.1% parasitism, respectively (Pawson and Gold, 1993). By contrast, Suiter et al. (1998) documented promising results, where they reported parasitism rates that ranged from 50 to 100% for releases made around treeholes infested with blattid pest cockroaches.